# Gene regulation by patterned electrical activity during neural and skeletal muscle development

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Patterned neural activity modifies central synapses during development and the physiological properties of skeletal muscle by selectively repressing or stimulating transcription of distinct genes. The effects of neural activity are mostly mediated by calcium. Of particular interest are the cellular mechanisms that may be used to sense and convert changes in calcium into specific alterations in gene expression. Recent studies have addressed the importance of spatial heterogeneity or of temporal changes in calcium levels for the regulation of gene expression.

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**Abbreviations**

| | |
|---|---|
| **CaM** | calmodulin |
| **CBP** | CREB-binding protein |
| **CRE** | cAMP response element |
| **CREB** | CRE-binding protein |
| **DRG** | dorsal root ganglion |
| **LTP** | long-term potentiation |
| **MAPK** | mitogen-activated protein kinase |
| **NCAM** | neural cell adhesion molecule |
| **NF-AT** | nuclear factor of activated T cells |
| **NMDA** | *N*-methyl-D-aspartate |
| **PDZ domain** | an amino acid repeat found in PSD-95, Dlg and ZO-1 proteins |
| **SRE** | serum response element |

## Introduction

The functional properties of neurons and skeletal muscle are plastic during development and in the adult, and they are modulated by afferent innervation. The ability of the nervous system to be remodeled with experience [1–3], and of skeletal muscles to adapt to different environmental demands [4,5], results, at least in part, from the regulation of gene expression in response to patterned electrical activity. For this reason, it is important to understand how distinct patterns of stimuli are coupled to specific changes in gene expression. Electrical activity regulates different transcription factors, which, in turn, regulate the expression of neurotransmitter receptors, ion channels, neurotrophic factors, cell adhesion molecules, cytoskeletal proteins, contractile proteins, and metabolic enzymes [6–11]. However, the number of these genes that have been shown to be sensitive to patterned activity, which is the emphasis of this review, is thus far limited.

Immediate early genes (IEGs) have been the focus of numerous studies in the nervous system regarding the coupling of electrical activity to transcription [12], but little is known of how the regulation of these factors results in changes in the expression of the structural proteins that modify neural and muscle function. In order to understand how activity contributes to the formation and phenotypic differentiation of the nervous system, it will be important to know how patterns of depolarization are sensed, decoded, and transduced into changes in the levels of transcription factors and other regulatory proteins that control the expression of genes encoding structural proteins. It is generally accepted that calcium is a major signaling molecule that transduces the activity into changes in cellular properties. But how are the different frequencies of depolarization and the calcium currents elicited by them decoded and then translated into distinct signals? Are different routes of calcium entry into the cell linked to distinct signaling pathways that respond differentially to patterned activity? Does the temporal and quantitative accumulation of calcium in different subcellular compartments activate distinct transduction pathways? Finally, how is transcriptional specificity achieved in response to depolarization patterns?

This review focuses on how expression levels of a gene or distinct members of gene families are regulated differentially by patterned activity. Although substantial progress has been made towards understanding signaling mechanisms that respond to patterned stimuli, we hope that it will be apparent from the review that this field, which is of central importance to understanding brain function, remains in its infancy.

## Regulation of muscle and neuronal properties by patterned activity

### Patterned activity differentially regulates skeletal muscle genes

Adult skeletal muscle is plastic. In response to changing extrinsic demands, muscle has the capacity to adapt by modifying its contractile and metabolic properties in response to different patterns of motoneuron activity [4,5,10,13]. Skeletal muscle historically has served as an excellent model to study activity-dependent plasticity and the modulation of gene expression in response to neural activity, because peripheral nerves are accessible for experimental manipulation and the resulting changes in muscle physiology can be easily quantified (e.g. force generation, twitch time). The importance of motoneuron innervation was first described by Eccles and colleagues [14], by demonstrating that fast-twitch muscles adopt slow-twitch properties when they are re-innervated ectopically by a nerve that normally innervates a slow muscle, and vice-versa. Activity, and not myotrophic factors released by the

nerve, were shown to be instructive to muscle because electrical stimulation of motor nerves [15] or denervated muscles [16] with patterns of stimuli that mimic natural motoneuron activity are sufficient to modify its contractile properties to the same extent as cross-innervation [17].

The transition of muscle properties evoked by patterned stimulation results from changes in the expression of numerous genes encoding specific fast and slow protein isoforms that determine the contractile and metabolic properties of muscles [4,10,13]. For example, depolarization of fast-twitch muscles with tonic, slow frequency (10 Hz) stimulation induces a sequential change in the synthesis of myosin heavy chain isoforms [18–20,21•], as well as changes in the calcium handling mechanisms of the cell. The latter include changes in the sarcoplasmic reticulum calcium-ATPase [22,23], dihydropyradine and ryanodine-sensitive calcium channels [23], and the calcium-sequestering proteins phospholamban [22,24] and parvalbumin [25].

It is important to emphasize, however, the role of cell lineage. Prior to motoneuron innervation, muscles can exhibit differences in the contractile proteins expressed [26], and neither cross-innervation [27] nor chronic stimulation of the denervated muscle fully change all the contractile properties of the fiber. Denervated fast-twitch extensor digitorum longus muscle and slow-twitch soleus muscle depolarized with the same activity patterns still manifest slight differences in their contractile properties [17]. Thus, the signaling pathways responding to patterned activity function in a cellular context that may be determined by the cell's developmental history or lineage. Evidence for slow and fast myoblast lineages that develop in the absence of motoneuron activity is rapidly accumulating ([26,28–30,31•]; see also the review by Hughes and Salinas [pp 54–64], in this issue, for a discussion of how these intrinsic differences may arise), suggesting that during muscle development the signaling mechanisms elicited by neural impulses interact or over-ride these inherited genetic programs.

The restricted expression of contractile proteins in specific muscle types and their regulation in response to neural activity is mostly controlled at the level of transcription [10]. Despite the fact that a large repertoire of muscle-specific genes regulated by patterned activity has been identified, little is known of the signaling pathways and transcription factors that couple the temporal changes in stimuli to specific changes in transcription. Direct proof of a role for calcium has not been formally established. These type of studies have been hindered because the full extent of muscle-type diversification does not occur *in vitro* and thus requires analysis *in vivo*.

One approach for mapping the pathways that lead from activity to fiber-type-specific transcription has been to use transgenic mice and somatic gene transfer (by the intramuscular injection of DNA constructs into adult muscles) to map DNA regulatory sequences conferring fiber-type specificity. The fiber-type-specific expression of the troponin I slow and fast isogenes, which requires motoneuron innervation and is differentially regulated by slow (10 Hz/10 s) and fast (100 Hz/1 s) bursts of activity [7,32], is conferred by enhancers of 128 and 148 base pairs, respectively [33]. Interestingly, both enhancers have conserved DNA motifs that bind to the transcription factors MyoD, MEF-2 and Sp1/CACC and that are required for transcription; these regulatory elements are also found in other muscle-specific genes. However, mutational analysis and the generation of chimeric enhancers — where the conserved motifs from the slow and fast enhancers have been swapped and tested in transgenic mice — demonstrate that although these sites are required for the enhancers to be active, they fail to restrict transcription to either slow or fast muscle fibers [7,34•]. Novel sequences that reside adjacent to the conserved motifs are necessary to direct transcriptional specificity of the troponin I genes (S Calvo, P Venepally, J Cheng, A Buonanno, unpublished data). Sequences required for the activity-dependent transcription of two slow myosin light chain genes were mapped by somatic gene transfer [35•,36•]. Both light-chain promoters share three of the elements found in the troponin I genes, but a sequence responsible for conferring the ability to respond to specific patterns of activity has not yet been found. The identification of these sites will be invaluable for determining how elements in the transcription regulatory complex interact to respond to patterned activity in a tissue-specific fashion.

## Frequency-dependent regulation of gene expression in neurons

Neural impulse activity can regulate a number of functional processes in the central and peripheral nervous systems, including neuronal phenotype [37–39], neurite outgrowth [40–42], axon fasciculation [43–45], synaptogenesis and remodeling [46], and activity-dependent changes in synaptic strength [2]. The genes involved in these forms of activity-dependent plasticity are largely unknown, but there are numerous examples of neuronal genes that can be regulated by impulse activity, including immediate-early genes [47] and genes that encode proteases [48,49], neurotrophins and neurotrophic factors [9], neurotransmitter receptors [50], cell adhesion molecules [44,45,51•], cell surface molecules [52], and novel membrane [40] and cytoskeletal [53] molecules.

As in muscle, the frequency or pattern of electrical impulses in neurons can be an important factor in activity-dependent gene regulation. High-frequency stimulation induces transcription of c-*fos*, c-*jun* and *jun*B in hippocampal neurons, but stimulus patterns inducing long-term potentiation (LTP) selectively trigger induction of *zif*268 [54]. Frequency-specific regulation of neuronal genes has also been described for some structural genes. Expression of the cell adhesion molecule L1 is downregulated by 0.1 Hz stimulation in dorsal root ganglion (DRG) neurons, but 1 Hz stimulation is without effect [44]. In contrast, N-cadherin mRNA is downregulated by both

0.1 Hz and 1 Hz stimulation in DRG neurons, whereas NCAM-180 expression is not altered measurably [51•].

Together, these results suggest that the pattern of neural impulse activity a neuron experiences can have significant functional effects that are dependent on the activation of the appropriate genes. Three examples illustrate this at the behavioral, cellular, and synaptic levels of organization, respectively. First, the conversion of short-term memory into long-term memory, which requires CREB-dependent gene expression [55], only takes place when training sessions are repeated at appropriate intervals. The same number of trials presented in one session are ineffective both in altering gene expression and in allowing memory [56]. A second example, myelination of axons, involves changes in expression of a large number of genes controlling this highly regulated interaction between neurons and glia [57]. Recent work shows that induction of myelination by Schwann cells is influenced by the specific temporal pattern of impulse activity in the axon, through changes in axonal expression of the cell adhesion molecule L1 [58•]. A third example of how activity can contribute to the synaptic level of organization comes from CA1 neurons of the hippocampus: brief synaptic activation at high frequency (100 Hz), or short high-frequency bursts repeated at 5 Hz intervals, induce LTP. In contrast, prolonged low-frequency stimulation (15 min at 1 Hz) depress the strength of the same synapses, a phenomenon referred to as long-term depression (LTD) [59]. Maintenance of LTP requires gene transcription [60] that is associated with MAPK [61] and CREB [62] phosphorylation.

## How is patterned activity sensed and decoded?

How specificity between stimulus and response is maintained within a broadly interactive network of intracellular signaling reactions and transcriptional regulatory processes is a general problem in cell biology. In excitable cells, however, the problem is compounded because membrane depolarization stimulates signaling pathways primarily through changes in intracellular free calcium levels, rather than through discrete receptors. How can different patterns of calcium flux activate different signaling pathways to the nucleus? The signaling mechanisms that confer stimulus–response specificity in cells could be divided into three general categories: discrete pathways, spatial heterogeneity, and temporal specificity.

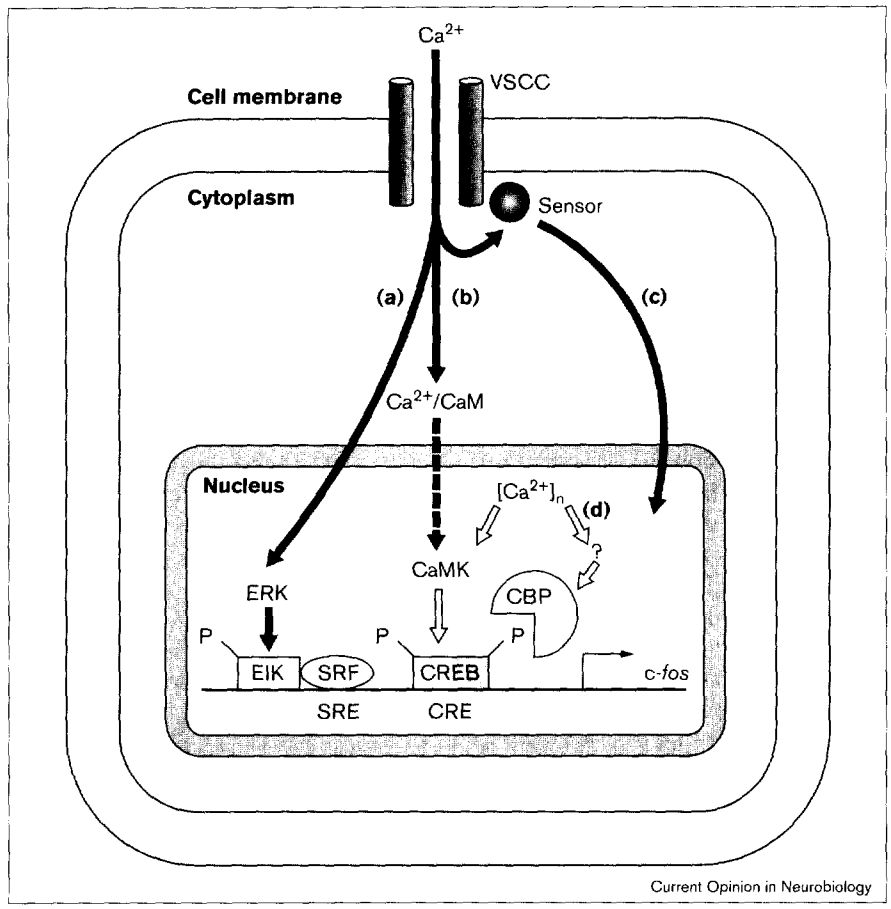
### Discrete pathways

Activation of specific receptors by appropriate ligands can stimulate discrete signaling pathways to regulate expression of target genes, but it is conceivable that the multiple points of convergence and divergence among signaling pathways, and the interactions among DNA-binding proteins, would undermine this specificity. For example, the transcription factor CREB mediates responses to both cAMP [63] and calcium [64] in some cells. Down-stream from calcium, signaling cascades can propagate from multiple calcium-sensitive kinases, including calmodulin (CaM) kinase II [12,65], CaM kinase IV [66] and MAPK [67], all of which are capable of phosphorylating CREB. Within the nucleus, the transcriptional apparatus also shows a high degree of interaction that would appear to further entangle signaling pathways from distinct stimuli to specific response genes. For example, the c-*fos* promoter contains two different regulatory sequences, the serum response element (SRE) and the cAMP response element (CRE), that were initially associated with trophic factor [68] and cAMP/calcium [63,69] responses, respectively. However, potential cross-talk between these two pathways and the combinatorial interactions among the DNA-binding proteins and transcriptional apparatus [70] complicate the separation of signaling pathways from different stimuli.

Such interactions between signaling pathways activated by either growth factors or membrane depolarization [71] could degrade response specificity. However, such interactions could also increase stimulus–response specificity by integrating multimodal stimuli within cells. A recent example is the regulation of the NR2C subtype of NMDA receptors, which requires both the activation of ErbB tyrosine kinase receptors by neuregulin and the activation of NMDA receptors by glutamate [72••]. Both of these stimuli can be provided by the mossy fiber inputs that innervate granule cells during development and that upregulate NR2C expression (A Buonanno, unpublished data). The requirement for conjoint activation of Trk tyrosine kinase and NMDA receptors has also been observed in the regulation of dendritic growth [73], but the genes mediating this response have not been identified. The fact that transcriptional enhancers function in a combinatorial fashion, and that the cooperative interaction of distinct DNA regulatory elements are often required to achieve transcriptional activation, is consistent with the idea that the temporal summation of calcium or synergy by co-activation of distinct pathways during synaptic transmission could be important for stimulus-dependent transcription.

Different frequencies or patterns of impulse activity can be encoded in the concentration of calcium produced in the cell [74,75•]. Higher levels of calcium could activate different intracellular signaling pathways than lower levels, and thereby activate different cellular substrates or genes (see [76]). The balance between calcium-regulated kinase and phosphatase activity also could be shifted by the level of calcium [76–78]. Transgenic mice overexpressing a constitutively active form of calcineurin, a calcium-dependent phosphatase, in the hippocampus support the idea of a temporal, activity-dependent balance between phosphorylation and dephosphorylation that regulates the transition from early-phase LTP to late-phase LTP [79••]. These calcineurin-overexpressing mice manifest deficits in spatial and visual recognition tasks requiring long-term memory when the number of training trials is low but perform as well as wild-type mice when the number of trials is increased [80•], suggesting that the amount of activity is important for the transition from early- to late-phase LTP.

**Figure 1**




Spatial heterogeneity of signaling pathways can provide gene expression specificity. The model depicts how distinct pools of cytoplasmic and nuclear calcium can in some cases signal differentially through SRE and CRE, respectively [84••,85••].
**(a)** Cytoplasmic calcium can activate transcription via the SRE and EIK [64,84••].
**(b,c)** The existence of calcium sensors, including CaM [86,87••], may reside in close association with receptors and voltage-sensitive calcium channels (VSCCs) at the subsynaptic membrane to signal from synapses to the nucleus. **(d)** In this model [84••,85••] nuclear calcium and CaM-dependent kinases (CaMKs) are necessary for the phosphorylation of CREB at Ser133 and the recruitment of the CREB-binding protein (CBP) in order to efficiently activate transcription of the *c-fos* gene.
ERK, extracellular signal regulated kinase.

The calcineurin-dependent pathway, acting through the transcription factor NF-AT (nuclear factor of activated T cells), has also been proposed to regulate the transcription of contractile genes expressed specifically in slow-twitch skeletal muscle in response to patterned activity [81•]. However, the NF-AT-binding site located in the enhancer of the slow troponin I gene — proposed in these studies to be the site conferring transcription specifically in slow muscles [81•] —has recently been shown not to be necessary for the expression of the troponin I enhancer in the slow-twitch muscles of transgenic mice [34•]. Further experiments will be needed to determine how the calcineurin/NF-AT pathway may regulate the fiber-type-specific expression of slow contractile genes, or their levels, in response to patterned activity.

## Spatial heterogeneity

Spatial heterogeneity can provide specificity between a stimulus and response when either cell surface receptors, signaling enzymes, or transcription factors are localized in distinct subcellular compartments. Indeed, the subcellular localization of transcription factors, such as NF-κB and NF-AT, is regulated by calcium [82,83]. Whether calcium influx controls transcription of *c-fos* through SRE or CRE can depend on whether calcium enters through NMDA channels or L-type calcium channels, because the signaling pathways associated with each mode of calcium entry are distinct and are distributed in different parts of the neuron [71]. Recently, two groups have reported on the differential regulation of immediate early gene expression [84••,85••] and CREB phosphorylation [86,87••] with respect to the spatially distinct subcellular localization of calcium in the cytoplasm versus nucleus [84••,85••], and the nucleus versus the submembranous compartment [86,87••]. Microinjection of an immobilized calcium chelator into the nucleus of AtT20 pituitary cells was used to demonstrate that distinct *cis*-acting elements in the *c-fos* promoter are differentially responsive to increases in cytoplasmic versus nuclear calcium after activation of L-type voltage-gated calcium channels [84••,85••]. Whereas increases in cytoplasmic calcium signal via the *c-fos* SRE, nuclear calcium signals through the CRE (see Figure 1). Interestingly, phosphorylation of CREB at Ser133 is necessary but not sufficient to mediate transcription via the CRE. Nuclear calcium and CaM kinase IV are required for the recruitment and activation of the CREB-binding protein (CBP), and the activation of CRE-mediated transcription [85••]. There is little evidence, however, that the nucleus represents a diffusion barrier to calcium or that differences in action potential

firing patterns result in disproportionate increases in cytoplasmic versus nuclear calcium in neurons [88,89••].

By contrast, the extent of CREB phosphorylation at Ser133 in dissociated hippocampal neurons differs with stimulus frequency and does not require nuclear calcium [86]. Deisseroth *et al.* [86] proposed the existence of a submembranous calcium sensor after observing that the amount of activity-dependent CREB phosphorylation varies when neurons are preloaded with either EGTA or BAPTA, which are predicted to differentially remove calcium based on their different rates of chelation. Calmodulin was proposed as a candidate for the calcium sensor located near the site of calcium entry. Activation of NMDA receptors and L-type voltage-sensitive channels leads to the rapid translocation of calmodulin to the nucleus and CREB phosphorylation at Ser133 [87••]. Together, these results indicate that signaling from the plasma membrane to the nucleus can be highly dependent on the subcellular compartmentalization of calcium and proteins in the signaling cascade. The recent demonstration of direct interactions between channels and receptors with other signaling molecules sequestered by PDZ-domain proteins emphasizes the importance of specialized microdomains that could sense localized changes of calcium and connect these to intracellular signal transduction pathways [90]. Indeed, a close association between the *Drosophila* photoreceptor TRP (transient receptor potential) calcium channel and a signaling complex that includes calmodulin, rhodopsin and phospholipase C is mediated via the PDZ-domain-containing protein INAD [91]. On the basis of these findings and those of RW Tsien's group [86•,87••], it is interesting to consider the possibility that PDZ-containing proteins at hippocampal synapses could closely associate L-type voltage-gated channels to calmodulin-or calmodulin-binding proteins to couple local calcium transients to pathways that signal to the nucleus.

The spatial distribution of cytoplasmic calcium in dendrites could be important for signal processing and gene expression in response to sensory stimulation. Changes in free calcium can be localized to subregions of the dendritic shaft [92] or confined to individual dendritic spines [93]. However, active sodium or calcium conductances can lead to global increases in dendritic calcium [94]. The influence of back-propagating action potentials on dendritic calcium transients varies with dendritic branching, pattern of neuronal activity, and physiological conditions [94]. Measurements using two-photon microscopy under normal physiological conditions have shown that the amplitude of dendritic calcium transients is proportional to the number of sodium action potentials induced by vibrissae stimulation, and that the concentration of calcium declines steeply with increasing distance from the soma [75•]. Thus, some patterns of synaptic activity or action potentials can be converted into spatial differences in calcium; presumably, this could activate different signaling pathways in distinct subcellular compartments.

### Temporal specificity

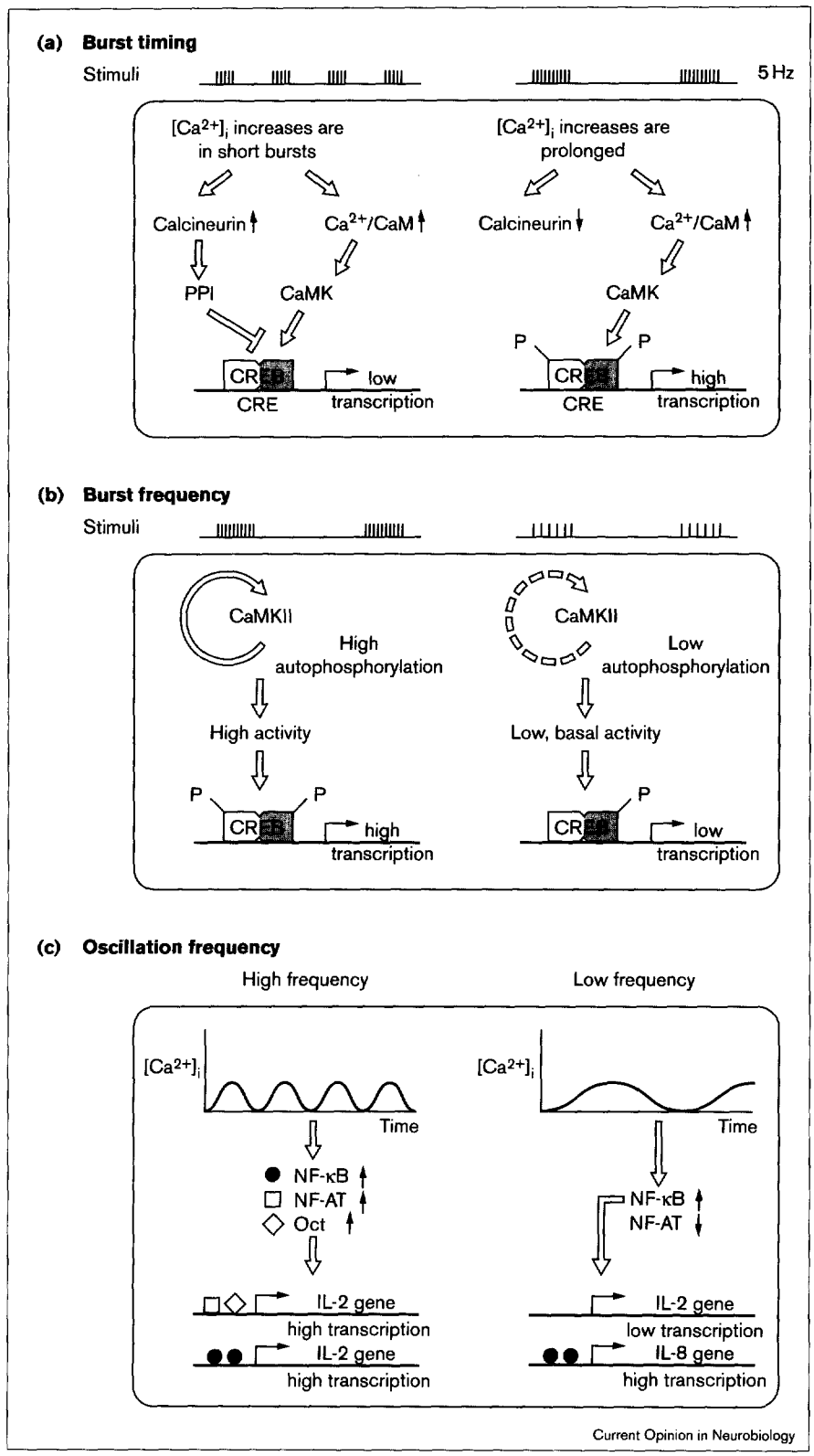
Another mechanism for signaling specificity would be provided if temporal patterns of calcium entry could be 'decoded' to selectively regulate gene expression. Measurements of intracellular calcium dynamics in mouse DRG neurons in response to action potentials have shown that some growth cone responses are correlated with the rate of calcium increase rather than the peak concentration of calcium [95]. In addition, in DRG neurons, c-*fos* mRNA levels are correlated with the interval of time between calcium influx induced by bursts of action potentials, not by the concentration of calcium [89••,96]. The latter experiments revealed three unexpected results: first, transcription of the c-*fos* gene did not require a sustained increase in cytoplasmic calcium; second, large increases in intracellular calcium are less effective in stimulating c-*fos* expression than small increases presented at shorter interburst intervals; and finally, c-*fos* expression is increased by stimuli that produced minimal, nearly undetectable changes in cytoplasmic calcium, provided the stimulus was repeated at appropriate temporal intervals. Similar results have been obtained from studying regulation of potassium channel maturation and neurite outgrowth in frog spinal cord neurons. These functions correlate with the frequency of calcium spikes and waves (2–15/h), not with the amplitude of the increase in cytoplasmic calcium [97].

Gene regulation in response to action potentials of very low frequencies have also been documented, where only minimal transient changes in cytoplasmic calcium would be generated. One action potential every 10 s is sufficient to stimulate c-*fos* expression in DRG neurons [96]. The same low-frequency stimulation lowers expression of the cell adhesion molecule L1, but higher-frequency stimulation or chronic depolarization with potassium-chloride are without effect [44]. These results illustrate the importance of the temporal dynamics of calcium entry, rather than calcium concentration, in regulating gene expression in response to action potentials in neurons.

## How can action potential patterns regulate different genes?

One hypothesis for how action potential patterns regulate different genes is that intracellular signaling reactions will propagate temporally varying stimuli differently depending upon the kinetic responses of the pathway [98]. For example, the kinetics of MAPK and CaM kinase activation and inactivation differ markedly [99], and should therefore respond preferentially to stimuli with temporal dynamics that favorably match the dynamics of each pathway. Recently, we have shown that different intracellular pathways regulating transcription of the c-*fos* gene can be activated selectively by patterns of action potentials that are favorably matched to the temporal dynamics of the signaling pathway [89••]. In these neurons, phosphorylation of CREB is rapid and sustained for several minutes, but phosphorylation of MAPK is more transient. Pulsed stimuli that were repeated at intervals

**Figure 2**




Several forms of temporal specificity provide mechanisms that could be utilized to selectively regulate gene expression in response to patterned activity. **(a)** The length of action potential bursts delivered at 5 Hz frequency have differential effects on the accumulation of calcium and on the balance of phosphatases (calcineurin, PP1) and CaM kinase (CaMK) activity in the cell [76,107]. This balance can differentially regulate CRE-mediated gene expression by influencing the phosphorylation dynamics of CREB. **(b)** The frequency of calcium stimuli affects the rate of calcium accumulation, which, in turn, affects the balance of CaMKII phosphorylation/dephosphorylation and the autocatalytic activity of the kinase. Higher-frequency calcium oscillations result in higher CaMKII autonomous activity [105••], which appears to be required for LTP [104•], presumably by affecting activity-dependent gene expression. **(c)** The frequency of calcium oscillations in non-neuronal cells differentially regulates the function of the transcription factors NF-κB, NF-AT and Oct, which have preferences for DNA-binding sites in the promoters of interleukin-8 (IL-8) and interleukin-2 (IL-2), thus selectively regulating the expression of downstream genes [110••,111••].

that were too long (e.g. 3–5 min) failed to allow levels of phosphorylated MAPK to accumulate, but did activate CREB at maximal levels. Consistent with the combinatorial requirement for multiple DNA-binding proteins and the basal transcriptional complex [70], maximal c-*fos* expression was obtained in response to pulsed patterns of action potentials that coordinately activated both the MAPK and CREB signaling pathways.

In response to action potential stimulation that produces either a large or a prolonged increase in intracellular calcium, the serine/threonine CaM kinase II [78] and the phosphatase calcineurin undergo changes in reaction kinetics (see [76]). This behavior may permit them to act as spike-frequency or stimulus-duration 'switches or detectors' that could activate specific signaling pathways regulating gene transcription in response to appropriate temporal features of impulse activity in neurons and muscles (see Figure 2). Autophosphorylation of CaM kinase II changes the calcium sensitivity and reaction kinetics (i.e. rate of calmodulin dissociation from CaM kinase II) to transmit signals from high-frequency stimuli (i.e. those producing higher levels of intracellular calcium) through the CaM kinase II pathway [78,100–103]. The importance of CaM kinase II autophosphorylation activity was recently shown *in vivo* using mice harboring a mutation in threonine-286 of CaM kinase II [104•], which blocks the autocatalytic activity of the enzyme. Mutant mice show impaired NMDA-dependent LTP and spatial learning deficits on the Morris water maze [104•].

Experiments performed with purified CaM kinase II immobilized to a rapid perfusion chamber have shown that different frequencies of calcium oscillations result in different levels of autonomous kinase activity [105••]. At low frequencies, the autonomy of the enzyme due to autophosphorylation is lower but increases sharply when stimuli are delivered at higher frequencies (Figure 2). The levels of kinase activity are also regulated by the subunit composition of the enzyme, which have different affinities for calmodulin [105••]. Such frequency-dependent regulation of CaM kinase II autophosphorylation needs to be demonstrated *in vivo* using natural stimuli because alternative models have been proposed [106].

The activity-dependent regulation of phosphatases can also modify the kinetics of signaling in response to calcium. A mechanism for detecting the duration of action potential bursts in dissociated hippocampal neurons has been proposed [76,107]. Longer duration bursts (e.g. 180 s versus 18 s at 5 Hz) resulted in inactivation of calcineurin and prolonged the period that phospho-CREB remained at elevated levels in the nucleus (Figure 2). This lead to elevated levels of c-*fos* and somatostatin gene expression, which are regulated via the CRE. Phosphatases (e.g. PP-1, PP-2B/calcineurin) also appear to play an important role in sustaining high levels of phospho-CREB in response to L-type calcium channel activity and in inducing c-Fos in developing striatal slice preparations [108].

Temporal segregation of intracellular signaling is not unique to neurons or muscle. Modulating intracellular calcium periodically by exposure to potassium-chloride or a calcium ionophore is more effective than sustained calcium influx in regulating prolactin gene expression in pituitary cells [109]. Two recent papers have addressed how calcium oscillations optimize the efficiency and specificity of gene expression in non-neuronal cells [110••,111••]; it is important to emphasize that the frequencies of intracellular calcium oscillations in these systems are considerably lower than the action potential frequencies observed in mature neurons and muscles. A calcium-clamp technique was used to regulate the frequency and amplitude of intracellular calcium in populations of T lymphocytes, which were treated with thapsigargin to deplete internal calcium stores, to analyze how distinct oscillation patterns regulate the expression of transcription factors that module interleukin-2 and interleukin-8 transcription [110••]. NF-κB was activated by low-frequency oscillations, whereas high frequencies were necessary to recruit NF-AT, Oct and NF-κB (see Figure 2). In turn, the promoters for the interleukin-8 and interleukin-2 genes demonstrated distinct preferences for the frequencies of intracellular calcium that correspond to their preferential regulation by NF-κB (low frequency) and NF-AT and Oct (higher frequency), respectively. The regulation of NF-AT by oscillation frequency was also observed by RY Tsien and colleagues [111••], who used a cell-permeant caged inositol 1,4,5-triphosphate (InsP3) activated by ultraviolet light to induce oscillations in intracellular calcium in a variety of cell lines by releasing calcium from internal stores. NF-AT-driven gene expression was found to be more effective if the changes of InsP3 and cytoplasmic calcium changed in waves, instead of being maintained at high steady-state levels. The time intervals between the peaks of cytoplasmic calcium levels were important for maximum NF-AT function. These studies emphasize the importance of the temporal changes of calcium, rather than changes in amount.

## Conclusions

Regulation of gene expression by specific patterns of impulse activity is essential for nervous system and skeletal muscle function. As experiments on transcriptional regulation in excitable cells moves from identifying the components of the system to observing how the components operate as a system in a dynamic state, the field is entering into an exciting new phase. Many properties that are essential for stimulus–transcription coupling may not be apparent from analysis of the system in a static state. Studies under dynamic conditions will bring us closer to understanding how the nervous system responds and adapts to changes in the environment and in its operation over time. A future challenge will be to identify the structural genes that are regulated by patterned activity that modifies neuronal plasticity, and to elucidate their mode of regulation. The integration of regulatory factors activated by activity patterns, and their interaction with transcription factors confined to specific cell types by lineage or development, provides further refinement for stimulus–transcription coupling.

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- • of special interest
- •• of outstanding interest


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